

**SELENIUM AND SELENOPROTEIN P1 LEVELS ARE RELATED TO PRIMARY OPEN-ANGLE GLAUCOMA**NIVOI SELENA I SELENOPROTEINA P1 POVEZANI SU  
SA PRIMARNIM GLAUKOMOM OTVORENOG UGLAMohammad Najafi<sup>1</sup>, Morteza Nouruzi Yeganeh<sup>1</sup>, Arezoo Miraftebi<sup>2</sup>,  
Reza Zarei<sup>3</sup>, Isa Noormohammadi<sup>1</sup><sup>1</sup>Biochemistry Department, Iran University of Medical Sciences, Tehran, Iran<sup>2</sup>Rasoul Akram Hospital, Iran University of Medical Sciences, Tehran, Iran<sup>3</sup>Farabi Eye Hospital, Tehran University of Medical Sciences, Tehran, Iran**Summary**

**Background:** Glaucoma is a highly prevalent eye disease related to optic nerve lesions and visual field defects. Primary Open-Angle Glaucoma (POAG) is a type of glaucoma that occurs frequently with unknown etiology. In this study, we investigated the serum levels of selenium, selenoprotein P1, glutathione, hemolysate glutathione peroxidase1 (GPx1) activity and aqueous humour selenium in POAG patients.

**Methods:** Ninety sex- and age-matched subjects (POAG patients; n=45 and, controls; n=45) with the controlled confounders (smoking, hypertension and alcohol beverages) were recruited on clinical histories and exams. The serum and aqueous humour selenium levels were measured using GFAAS technique. The serum selenoprotein P1 level was assayed with the ELISA method. The hemolysate GPx1 activity and serum reduced glutathione level were also measured using known colorimetric techniques.

**Results:** The serum selenium ( $P=0.01$ ) and selenoprotein P1 ( $P<0.001$ ) levels were significantly high in POAG patients. Furthermore, the aqueous humour selenium level was significantly high among patients as compared to controls ( $64.68 \pm 13.07$  vs.  $58.36 \pm 13.76$  ng/mL,  $P=0.02$ ). The results did not show a significant difference ( $P=0.36$ ) in the hemolysate GPx1 activity between the groups. The cut-off points for intraocular pressure (IOP) and serum selenoprotein P1 parameters were estimated to be 39 mmHg (sensitivity 97.5%; 1-specificity 6.5%) and 188  $\mu$ g/mL (sensitivity 93.5%; 1-specificity 14%), respectively.

**Kratak sadržaj**

**Uvod:** Glaukom je veoma često oboljenje oka koje je povezano sa lezijama na očnom živcu i oštećenjima vidnog polja. Primarni glaukom otvorenog ugla (PGOU) tip je glaukoma sa često nepoznatom etiologijom. U ovoj studiji ispitivani su serumski nivoi selena, selenoproteina P1, glutathiona, aktivnost hemolizata glutation-peroksidaze1 (GPx1) i selen u očnoj vodici kod pacijenata sa PGOU.

**Metode:** Na osnovu kliničke istorije i pregleda, obuhvaćeno je 90 subjekata odgovarajućeg pola i starosne dobi (pacijenti sa PGOU; n=45 i kontrolni subjekti; n=45) sa kontrolisanim varijablama (pušenje, hipertenzija i alkoholna pića). Nivoi selena u serumu i očnoj vodici izmereni su pomoću tehnike GFAAS. Nivo selenoproteina P1 u serumu određen je metodom ELISA. Aktivnost hemolizata GPx1 i nivo redukovanog glutathiona u serumu takođe su mereni poznatim kolorimetrijskim tehnikama.

**Rezultati:** Serumski nivoi selena ( $P=0.01$ ) i selenoproteina P1 ( $P=0.001$ ) bili su značajno viši kod pacijenata sa PGOU. Uz to, nivo selena u očnoj vodici bio je značajno viši kod pacijenata nego u kontrolnoj grupi ( $64,68 \pm 13,07$  prema  $58,36 \pm 13,76$  ng/mL,  $P=0,02$ ). Rezultati nisu otkrili značajnu razliku ( $P=0,36$ ) u aktivnosti hemolizata GPx1 između grupa. Određene su *cutoff* vrednosti za intraokularni pritisak (IOP) i parametre serumskog selenoproteina P1: 39 mmHg (osetljivost 97,5%; 1-specifičnost 6,5%), odnosno 188  $\mu$ g/mL (osetljivost 93,5%; 1-specifičnost 14%).

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**Conclusions:** The results also showed the selenium and selenoprotein P1 levels relate to intraocular pressure. We suggested serum selenoprotein P1 and intraocular pressure are more sensitive factors for the primary screening of POAG.

**Keywords:** selenium, selenoprotein P1, glutathione peroxidase 1, glutathione, glaucoma

## Introduction

Glaucoma is an eye disease related to the optic nerve damage and visual field loss (1). It is the second leading cause of blindness after cataract. Based on the trabecular meshwork (TM) obstruction, glaucoma can clinically be observed in two forms, Open-Angle Glaucoma (POAG) and Primary Closed-angle glaucoma (PACG). Primary Open-Angle Glaucoma (POAG) is a type that frequently occurs in the United States; however, a shift is considered in the demographic and geographic characteristics of the patients (2). The high prevalence of glaucoma is also reported in Asian countries (3). In PACG, the iris obstructs the trabecular meshwork in the angle of the eyes, whereas in POAG, the trabecular meshwork seems to be open and unobstructed by the iris (4). Although the POAG etiology is unknown, ganglion cell death is proposed to be due to high eye pressure, decreased blood flow to the optic nerve head, inflammatory events, and increased nitric oxide and glutamate levels (5, 6). In addition to the above factors (7), other agents such as high selenium in bodily fluids, molecular genetics and some demographic parameters have also been suggested to be involved in glaucoma, but so far the evidence is inconclusive (8–10).

Selenium is present in body fluids (11) and is known as a functional trace element in the human lens (12). Some studies have reported the association between serum selenium level and glaucoma (13) and have shown that selenium overload within aqueous humour may cause a decrease in trabecular meshwork cell adhesion and activation of apoptotic signaling pathways (14, 15). Based on these reports, we evaluated the levels of selenium, selenoprotein P1 and some antioxidant parameters to test the hypothesis which suggests POAG may be related to a high selenium level.

## Methods

### Subjects

The sex and age-matched subjects were recruited upon clinical exams. The POAG patients (n=45) were excluded for secondary glaucoma, congenital glaucoma and PACG and were candidates for trabeculectomy or phacoemulsification. Controls (n=45) had clinically normal optic nerves and intraocular pressure which did not contribute to POAG risk. The study was approved by the university ethics commit-

**Zaključak:** Rezultati su takođe pokazali da su nivoi selena i selenoproteina P1 povezani sa intraokularnim pritiskom. Ukazali smo na to da su selenoprotein P1 u serumu i intraokularni pritisak osjetljiviji faktori za primarni skrining za PGOU.

**Ključne reči:** selen, selenoprotein P1, glutation peroksidaza 1, glutation, glaukom

tee and informed consent was obtained from all subjects.

### Samples

Two blood specimens (each 5 mL) were collected in the blood tubes. The clotted and EDTA blood tubes were centrifuged at 3000 g (15 min) and the serum and plasma were separated and stored at  $-80^{\circ}\text{C}$ . The remaining red pellets were washed three times with normal saline (NaCl, 0.9%) and were diluted with distilled water for preparation of erythrocyte hemolysate (1: 1 V/V).

### Serum and aqueous humour selenium levels

The serum and aqueous humour selenium values were measured using Graphite Furnace atomic absorption spectrometry (GFAAS). Five mL  $\text{HNO}_3$  (16 mol/L) was added to 2.5 mL serum and then slowly heated up to  $140^{\circ}\text{C}$  (20 min). The above stage was repeated with an acidic solution containing 2.5 mL  $\text{H}_2\text{SO}_4$  (18 mol/L) and 1 mL  $\text{HClO}_4$  (11.6 mol/L). Then, 5 mL HCl (5 mol/L) was added after cooling and 10  $\mu\text{L}$  from it was injected into a Parker Elmer atomic spectrometer (Mercury Hydride system). The aqueous humour samples were directly injected into the system.

### Selenoprotein P1

The serum selenoprotein P1 concentration was measured with the ELISA method (Antibodies Online, Cusabio Biotech Co). According to the protocol, 5  $\mu\text{L}$  sera were diluted and incubated with reagents. Then, the serum selenoprotein P1 concentration was calculated on the standard curve (Boot strapping, 100).

### Glutathione (GSH)

The serum sample was mixed with the precipitating reagent (metaphosphoric acid (100 g/L) and phosphate buffer (0.3 mol/L, pH 7.5)) and centrifuged at 2000 g (2 min). 0.2 mL phosphate buffer (0.3 mol/L, pH 7.5) was mixed with 200  $\mu\text{L}$  supernatant and the sample absorption (A) was identified at 412 nm (Beutler method).

$\text{Glutathione concentration (nmol/mL)} = (A/\text{molar extinction coefficient (GSH)}) \times \text{Dilution coefficient}$

### Hemolysate GPx1 activity

The hemolysate GPx1 enzyme was coupled to glutathione reductase (GR) and its activity was normalized with the hemolysate hemoglobin (Hb) concentration (Zist-Shimi Co). According to the method, 20  $\mu$ L of the hemolysate was added to 400  $\mu$ L of reactive reagent (8 mL phosphate buffer (100 mmol/L, pH 7.4), 4 mL GR (5000 U/L), 2 mL GSH (2.5 mmol/L) and 2 mL NADPH (2.5 mmol/L)). Then, 20  $\mu$ L TBHP (25 mmol/L) was added to the previous solution and the absorbance changes ( $\Delta$ OD) were calculated after an incubation period (3 min) (16).

$GPx1 \text{ activity (U/g Hb)} = ((\Delta OD \times \text{dilution coefficient}) / \text{molar extinction coefficient (NADPH)}) / 10 \times \text{Hb (g/dL)}$

### Total Antioxidant Capacity (TAC)

According to the method, ABTS (2, 2'-azino-di (3-ethylbenzthiazoline-6-sulfonate) is converted to  $ABTS^{\bullet+}$  (oxidized ABTS, green) by  $H_2O_2$ . Then, linear decolorization follows in the presence of serum antioxidants. 200  $\mu$ L reagent 1 (Buffer acetate 0.4 mol/L, pH 5.8) was mixed with the serum sample (5  $\mu$ L) and then its absorbance (660 nm;  $OD_1$ ) was taken as sam-

ple blank. Afterwards, reagent 2 ( $ABTS^{\bullet+}$ , buffer acetate 30 mmol/L, pH 3.6) was added to the previous mixture and second absorbance ( $OD_2$ ) was taken after an incubation period (5 min). The results were reported on the basis of Trolox calibration.

### Statistics

Data was analyzed using a statistical software package (SPSS 18.0, Chicago). The parameters were evaluated using Student-t and Chi square tests between control and patient subjects. ROC curve was also used to identify the cutoff values. Linear regression analyses were performed and tested between some parameters. P value less than 0.05 was considered to be significant.

### Results

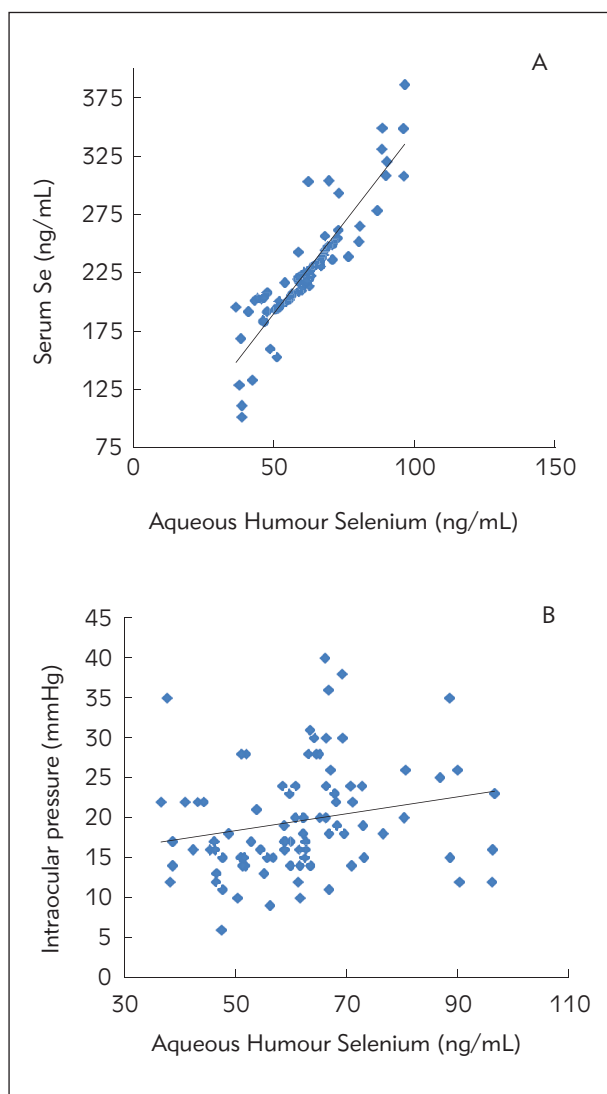
The age ( $P=0.2$ ) and sex ( $P=0.18$ ) parameters and some confounders such as systolic blood pressure ( $P=0.76$ ), diastolic blood pressure ( $P=0.59$ ) and smoking ( $P=0.82$ ) showed no significant differences between control and patient subjects. However, significant differences were observed in intraocular pressure (IOP) and central corneal thickness ( $P<0.0001$ ) (Table I).

**Table I** Characteristics of the study population.

Parameter	Control (n=45)	Patient (n=45)	P-Value
Sex (male/female)	26/19	32/13	0.18
Age (years)	67.91 $\pm$ 8.05	70.04 $\pm$ 7.71	0.20
Body Mass Index (kg/m <sup>2</sup> )	25.27 $\pm$ 4.11	25.31 $\pm$ 3.96	0.95
Systolic Blood Pressure (mmHg)	128.00 $\pm$ 14.55	128.89 $\pm$ 13.35	0.76
Diastolic Blood Pressure (mmHg)	77.32 $\pm$ 7.80	78.22 $\pm$ 7.76	0.59
Smoking (yes/no)	11/34	10/35	0.82
Intraocular pressure (mmHg)	14.40 $\pm$ 2.71	24.85 $\pm$ 5.67	<0.0001
Central corneal thickness (microns)	247.50 $\pm$ 172.09	427.60 $\pm$ 233.08	<0.0001

**Table II** Biochemical parameters.

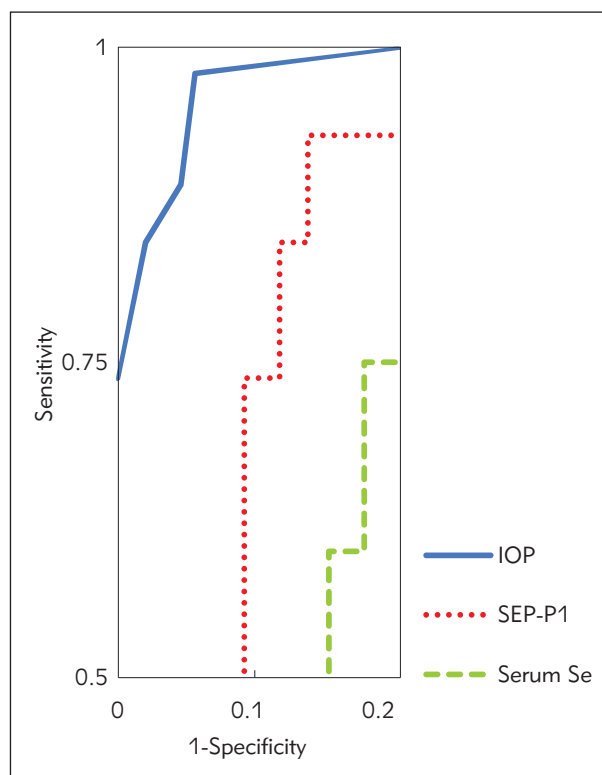
Parameter	Control (n=45)	Patient (n=45)	P-Value
Aqueous humour total antioxidant capacity (mmol/L Trolox equivalent)	277.30 $\pm$ 80.60	281.51 $\pm$ 112.22	0.84
Aqueous humour selenium (ng/mL)	58.36 $\pm$ 13.76	64.68 $\pm$ 13.07	0.02
Serum selenium (ng/mL)	213.57 $\pm$ 48.70	237.71 $\pm$ 43.49	0.01
Serum selenoprotein P1 (mg/mL)	13.90 $\pm$ 10.47	46.68 $\pm$ 34.38	<0.001
Serum glutathione (nmol/mL)	3.78 $\pm$ 1.49	4.43 $\pm$ 1.46	0.045
Hemolysate glutathione peroxidase1 (U/g Hb)	81.58 $\pm$ 25.39	76.37 $\pm$ 29.28	0.36



**Figure 1** Linear correlation (A) between aqueous humour selenium and serum selenium, (B) between aqueous humour selenium and intraocular pressure.

Table II shows that the selenium level increases significantly in the aqueous humour of POAG patients ( $P=0.02$ ). Furthermore, the serum selenium ( $P=0.01$ ) and selenoprotein P1 ( $P<0.001$ ) levels, similar to the aqueous humour selenium, increased significantly among patients. In contrast, the results did not show significant differences for the glutathione peroxidase1 (GPx1) activity ( $P=0.36$ ) and the total antioxidant capacity (TAC) ( $P=0.84$ ). The serum glutathione concentration was slightly increased in patients ( $P=0.045$ ) (Table II).

The results also showed that aqueous humour selenium had significant linear correlations with the serum selenium ( $R^2=0.81$ ,  $P=0.0001$ ) and intraocular pressure ( $R^2=0.75$ ,  $P=0.045$ ) (Figure 1 A and B). Based on the ROC curve (Figure 2), we estimated



**Figure 2** ROC curve. Intraocular pressure (IOP), serum selenoprotein P1 (SEP-P1) and selenium (Se) cutoff points are estimated on clinical sensitivity and 1-specificity (false positive).

the selenium ( $P=0.00$ ; Area 0.744, CI 0.633–0.855), selenoprotein P1 ( $P=0.00$ ; Area 0.879, CI 0.797–0.961) and intraocular pressure ( $P=0.00$ ; Area 0.989, CI 0.975–1.003) cutoff points and found that the serum selenoprotein P1 and intraocular pressure have higher quality characteristics for the primary screening of POAG. The intraocular pressure (IOP) and selenoprotein P1 (SEP-P1) cutoff points were 39 mmHg (sensitivity 97.5%; 1-specificity 6.5%) and 188  $\mu\text{g/mL}$  (sensitivity 93.5%; 1-specificity 14%), respectively.

## Discussion

In this study, the levels of selenium, selenoprotein P1, glutathione and GPx1 activity were evaluated on the basis of a long-term selenium supplementation study (Nutritional Prevention of Cancer; NPC) that showed a potential association between selenium and incidence of glaucoma. In agreement with the NPC results, our findings revealed that the body's selenium level may be one of the causal factors in the development of POAG. Some authors, however, did not find significant associations between selenium and glaucoma, but they suggested a higher risk for incidence of glaucoma as selenium level elevates in the aqueous humour (17).

In spite of laboratorial and biological variations, the results showed an association between POAG and body's selenium. The study also revealed a significant association between POAG and the serum selenoprotein P1 level, so that the ROC curve showed that it may be a more sensitive factor than the serum and aqueous humour selenium levels. These results pointed out that the continuous diffusion of serum selenium into aqueous humour leads to selenium overload within the MT cells. The increase of cellular selenium higher than the normal range could change the oxidant balance within the MT cells and may trigger the apoptotic signals (14). Coneley et al. showed that the selenium overload within TM cells may affect outflow resistance, routine phagocytic function and extracellular matrix turnover (18). The oxidant balance is inversely related to the antioxidant enzyme activities because some oxidant and antioxidant substances are known as the enzyme substrates or products. Thus, cellular involvement of selenium without the consideration of selenoenzymes may describe the TAC results between control and patient subjects.

Despite the increase in the body's selenium concentration, the GPx1 activity was not high in POAG patients. In agreement with other studies (19, 20), which showed maximum GPx1 activity occurs with

selenium values higher than 100 ng/mL, we found that the serum selenium concentrations were higher than 213 ng/mL.

The results also showed that the intraocular pressure higher than 39 mmHg and serum selenoprotein P1 levels higher than 188 µg/mL can be considered as cutoff points in the primary screening of POAG; however, these results must be confirmed in other studies.

## Conclusion

We found the aqueous humour selenium level is correlated to the serum selenium concentration. The intraocular hypertension also had a correlation with the selenium and selenoprotein P1 levels, so that the serum selenoprotein P1 level and intraocular hypertension are suggested as more sensitive factors for the primary screening of POAG.

## Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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